Genetic Linkage in Soybean: Classical Genetic Linkage Groups 6 and 8

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ABSTRACT

Mapping mutants of classical linkage groups 6 and 8 (CLGs 6 and 8) in soybean [*Glycine max* (L.) Merr.] can enhance the identification of important agronomic traits. Genetic data suggest that CLGs 6 and 8 may belong to the same linkage group. Our objectives were to determine if CLGs 6 and 8 are the same or different linkage groups and to determine the gene order. Genotypes containing mutants of CLGs 6 and 8 and mutants of other CLGs were crossed in various combinations. Data for the different characters were collected from F_2 populations and $F_{2:3}$ families. Recombination values confirmed that CLG 6 characters, Df_2 and Y_{11} were linked ($R = 27.0 \pm 5.9$), Df_2 was linked to Ms_1 (CLG 8) ($R = 24.8 \pm 1.2$) and to W_1 (CLG 8) ($R = 36.4 \pm 1.3$), and Y_{11} was linked to Ms_1 ($R = 31.7 \pm 1.4$). F_2 data suggested that Y_{11} segregated independently of W_1, while $F_{2:3}$ data indicated the two were linked ($R = 38.4 \pm 3.2$). Our data indicated that CLGs 6 and 8 belong to the same linkage group, which is molecular linkage group F (MLG F), and chromosome 13. Y_{11} and Adh_1 are at the ends of the chromosome segment studied, and Y_{23} is located between Ms_6 and St_5. Recombination values among the other loci of CLG 8, and between them and loci of other CLGs were consistent with published values. This information will be useful in the reassignment of CLGs, ordering of loci, and will enhance molecular genetic linkage mapping in soybean.

Soybean classical linkage group 8 (CLG 8), currently defined by seven gene loci (*Adh*$_1$, *Ms*$_1$, *Ms*$_6$, *St*$_5$, *W*$_1$, *Wm*, and*Y*$_{23}$), has been assigned to the nucleolus-organizing region (NOR) or satellite chromosome (Sadanaga and Grindeland, 1984), which was identified as chromosome 13 (Singh and Hymowitz, 1988). Through molecular mapping, 18 of the 20 classical linkage groups have been associated with a molecular linkage group. Cregan et al. (1999) assigned the flower color locus, *W*$_1$, to molecular linkage group F (MLG F), which enabled the integration of CLG 8 with MLG F. However, CLGs6 has not been associated with a molecular linkage group. Mutants on CLGs 6 and 8 could be associated with yield or seed weight quantitative trait loci (QTL) (Mansur et al., 1993; Orf et al., 1999), and *Adh1* could be associated with flooding tolerance QTL (VanToai et al., 2001). Therefore mapping mutants of CLGs 6 and 8 can enhance the identification of genes affecting important agronomic traits.

Factors such as the type of genetic data, environment, genetic background, genotype × environment interaction, and the linkage phase can influence recombination and result in a range of recombination values. The differences between recombination values obtained from backcross data, F_2 populations, and $F_{2:3}$ families have been documented (Allard, 1956; Haldane, 1919; Immer, 1930, 1934; Mather, 1936). Recombination values calculated from coupling data can differ significantly from those calculated from repulsion data (Butler, 1968; Immer, 1930, 1934; Mather, 1951).

Weiss (1970) reported a recombination value of 12.1 ± 0.7 between the Y_{11} and Df_2 loci that established soybean CLG 6. The first reported study involving loci of classical CLG 8 was by Palmer (1976), for Ms_1 and W_1. However, many of the soybean studies looked at linkage between two loci at a time. This manner of studying linkage can result in a range of significantly different map distances due to factors mentioned earlier. Variation in recombination values in soybean has been documented (Hildebrand et al., 1980; Kiang et al., 1985; Palmer et al., 1998b; Pfeiffer, 1993; Pfeiffer and Vogt, 1990; Yu and Kiang, 1990). While this can result in the assignment of loci to linkage groups, it makes the unambiguous assignment of gene order difficult. Palmer and Chen (1998) classified $F_{2:3}$ families and reported recombination values of 24.7 ± 2.2 for Df_2 -Y_{11}, 35.3 ± 3.0 for W_1 - Ms_1, 40.7 ± 2.8 for Df_2 - Ms_1, 42.1 ± 4.3, for Y_{11} - W_1, and 39.3 ± 5.3 for Y_{11} - Ms_1. On the basis of classification of F_2 populations, Mahama and Palmer (1998) reported recombination values of 37.1 ± 2.4 for Df_2 - W_1, and 28.5 ± 2.5 for Df_2 - Ms_1. These reports suggest that classical linkage groups 6 and 8 may be the same linkage group. Although recombination values of various loci pair vary, they are in general agreement in loci placement, hence the consensus classical linkage map of Palmer and Hedges (1993). Though a consensus map has been constructed, the gene order of some loci of CLG 8, and the independence of CLG 6 and CLG 8 is not confirmed. Our objectives were to determine if CLGs 6 and 8 are the same or different linkage groups, and to determine the gene order.

MATERIALS AND METHODS

In this study, genetic marker types were constructed that comprised various combinations of mutant loci of classical linkage groups 6 and 8, as well as mutant loci of the other linkage groups. The majority of the loci combinations were

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Abbreviations: CLG(s), Classical Linkage Group(s); MLG, Molecular Linkage Group; EC, Enzyme Commission.

Table 1. Phenotypic description of alleles of soybean genes studied to evaluate classical genetic linkage groups 6 and 8.

Gene	Classical linkage group†	Phenotype	Reference
T	1	Tawny pubescence	Piper and Morse (1910), Woodworth (1921)
t	1	Gray pubescence	
Df_2	6	Normal	Porter and Weiss (1948), symbol by Byth and Weber (1969)
df_2	6	Dwarf	
Y_{11}	6	Normal (heterozygote has greenish yellow leaves)	Weber and Weiss (1959)
y_{11}	6	Lethal yellow	
Adh_1	8	ADH band 1 present	Gorman and Kiang (1978), Kiang and Gorman (1983)
adh_1	8	ADH band 1 absent	
Ms_1	8	Fertile	Brim and Young (1971), Boerma and Cooper (1978), Palmer et al. (1978)
ms_1	8	Male sterile	
Ms_6	8	Fertile	Skorupska and Palmer (1989)
ms_6	8	Male sterile	
St_5	8	Fertile	Palmer and Kaul (1983)
st_5	8	Desynaptic sterile	
W_1	8	Purple flower	Takahashi and Fukuyama (1919), Woodworth (1923)
w_1	8	White flower	
Wm	8	Purple flower	Buzzell et al. (1977)
wm	8	Magenta flower	
Y_{23}	8	Normal	Palmer et al. (1990)
y_{23}	8	Leaves yellowish-white and necrotic	
Y_9	14	Normal	Probst (1950)
y_9	14	Bright greenish yellow	
Y_{10}	–	Normal	Probst (1950)
y_{10}	–	Greenish yellow seedlings	
Y_{18}	–	Normal	Peterson and Weber (1969)
y_{18}	–	Near-lethal yellow	

† Y_{10}, and Y_{18}, are not assigned to any linkage group at this time.

in coupling phase linkage. To obtain segregating populations, standard cross-pollination techniques (Walker et al., 1979) were used. Male-sterile plants of the genotype ms_1ms_1 and ms_6ms_6 were used as the female parents. Fertile plants (of genotype St_5st_5) in segregating families, identified by progeny testing, were used as pollen parents. F_2 seeds were produced from F_1 plants grown at the University of Puerto Rico/Iowa State University soybean nursery. The soil type is a very-fine, kaolinitic, isohyperthermic Typic Hapludox. Data for the different characters were collected from F_2 populations and $F_{2:3}$ families grown at the Bruner Farm near Ames, IA. The soil type is a Clarion-Nicollet Loam soil type (fine-loamy, mixed, superactive, mesic, Typic Hapludoll and fine-loamy, mixed, supeactive. mesic Aquic Hapludoll). All loci express complete dominance except the Y_{11} locus, which expresses incomplete dominance. Homozygous recessive ($y_{11}y_{11}$) plants are lethal, dying shortly after germination.

Segregation data for the alcohol dehydrogenase (EC 1.2.3.4) locus were obtained from F_2 seeds following the starch gel electrophoresis procedure of Cardy and Beversdorf (1984) with modifications (Mahama et al., 1995). Seedlings from sampled seeds were transplanted to the field at the Bruner Farm, in rows 1 m apart, with 0.45 m between seedlings, and segregation data collected for the other traits. Family rows were classified for flower color and plant color 6 wk after planting. At maturity F_2 plants were visually classified on the basis of pod set, as fertile (mostly three-seeded pods at all nodes) or sterile (no pods or occasional out-crossed pods at a few nodes). Dwarf (df_2df_2) and yellow ($y_{23}y_{23}$) plants among tall green plants in the field are difficult to classify accurately for fertility–sterility because they set few or no pods. Mature flower buds (with petals showing just above the sepals) were collected from each plant separately and placed into vials containing 70% (v/v) ethanol. The anthers were squashed and pollen dispersed in a drop of 1% (v/v) I_2KI solution (Jensen, 1962). Plants were classified as fertile (Ms_1-) or sterile (ms_1 ms_1) by counting pollen grains stained with I_2KI at ×100 magnification. At maturity, F_2 plants were individually threshed. $F_{2:3}$ progenies from self-pollination of F_2 plants were grown the following season. Approximately 50 to 60 seeds per progeny row were planted in 3-m-long rows, spaced 0.7 m apart. Progeny rows were classified for segregation of the various traits. Phenotypic descriptions of the marker loci used in this study are presented in Table 1. List of crosses made is shown in Table 2.

Data Analyses

Kramer and Burnham (1947) presented maximum likelihood estimation coefficients for calculating linkage intensity values and combining linkage intensity values from backcross, F_2, and $F_{2:3}$ genetic data for repulsion phase linkage, following the method of scoring by Fisher (1946). Making the necessary substitutions for recombination value and sign change, we derived the appropriate coefficients for coupling phase linkage. Coefficients were entered into a spreadsheet and used to calculate chi-square values to test independence of loci pair, and the corresponding recombination values as percentage recombination. Coupling, repulsion, F_2, and $F_{2:3}$ data were analyzed separately. Wherever applicable for any loci pair, F_2 and $F_{2:3}$ coupling and repulsion data were combined and analyzed following the method of scoring (Fisher, 1946). Similarly, recombination values between the Y_{11} locus and the other loci were calculated following the method of Weiss (1970) for repulsion data. Appropriate coefficients were derived for F_2 and $F_{2:3}$ coupling data as shown below by means of appropriate maximum likelihood estimation coefficients derived following the method of Mather (1935, 1951). The maximum likelihood estimator for recombination frequency is the value of p that would satisfy the equation below.

The F_2 phenotypic classes involving Y_{11} and gene loci are $a = Y_{11}Y_{11}$ A-, $b = Y_{11}y_{11}$ A-, $c = Y_{11}Y_{11}$ aa, and $d = Y_{11}y_{11}$ aa, in a 3:6:1:2 ratio, where A-, is either W_1-, or Ms_1- or Df_2-, or T- and aa is either w_1w_1, or ms_1ms_1, or df_2df_2 or tt.

The expectations of these classes are, respectively,

Table 2. List of soybean crosses used to generate segregation populations for evaluating linkages between classical linkage groups 6 and 8.

Cross number	Genotypes
1	$w_1w_1df_2df_2Y_{11}y_{11}ms_1ms_1 \times W_1W_1Df_2Df_2Y_{11}Y_{11}Ms_1Ms_1$
2	$W_1W_1Df_2Df_2Y_{11}Y_{11}$ *tt* $\times w_1w_1df_2df_2Y_{11}y_{11}TT$
3	$w_1w_1adh_1adh_1ms_1ms_1$ *TT* $\times W_1W_1Adh_1Adh_1Ms_1Ms_1$ *tt*
4	$w_1w_1adh_1adh_1ms_6ms_6 \times W_1W_1Adh_1Adh_1Ms_6Ms_6$
5	$w_1w_1adh_1adh_1y_{23}y_{23}$ *TT* $\times W_1W_1Adh_1Adh_1Y_{23}Y_{23}$ *tt*
6	$W_1W_1ms_1ms_1 \times w_1w_1Ms_1Ms_1$
7	$w_1w_1Y_9Y_9ms_1ms_1$ *tt* $\times W_1W_1y_9y_9Ms_1Ms_1$ *TT*
8	W_1W_1 *WmWmms*$_1$*ms*$_1 \times w_1w_1$ *wmwmMs*$_1$*Ms*$_1$
9	$w_1w_1Y_{11}y_{11}ms_1ms_1$ *TT* $\times W_1W_1Y_{11}Y_{11}Ms_1MS_1$ *tt*
10	$w_1w_1St_5St_5Ms_1ms_1 \times W_1W_1$ *St*$_5$*st*$_5$*Ms*$_1$*Ms*$_1$
11	$w_1w_1St_5St_5Ms_6Ms_6 \times W_1W_1$ *St*$_5$*st*$_5$*Ms*$_6$*ms*$_6$
12	$w_1w_1Ms_1ms_1Ms_6Ms_6 \times W_1W_1$ *Ms*$_1$*Ms*$_1$*Ms*$_6$*ms*$_6$
13	$w_1w_1y_{23}y_{23}ms_1ms_1 \times W_1W_1$ *Y*$_{23}$*Y*$_{23}$*Ms*$_1$*Ms*$_1$
14	$w_1w_1y_{23}y_{23}ms_6ms_6 \times W_1W_1$ *Y*$_{23}$*Y*$_{23}$*Ms*$_6$*Ms*$_6$
15	$Df_2Df_2ms_1ms_1 \times df_2df_2Ms_1Ms_1$
16	$W_1W_1Y_{10}Y_{10}ms_1ms_1 \times w_1w_1y_{10}y_{10}Ms_1Ms_1$
17	$Y_{18}Y_{18}ms_1ms_1 \times y_{18}y_{18}Ms_1Ms_1$
18	$w_1w_1y_{23}y_{23}St_5st_5 \times W_1W_1$ *Y*$_{23}$*Y*$_{23}$ *St*$_5$*St*$_5$
19	$w_1w_1adh_1adh_1$ *St*$_5$*St*$_5 \times W_1W_1Adh_1Adh_1$ *St*$_5$*st*$_5$
20	$w_1w_1df_2df_2Y_{11}y_{11}Ms_1ms_1 \times W_1W_1Df_2Df_2Y_{11}Y_{11}Ms_1Ms_1$
21	$w_1w_1TTEpEpms_1ms_1 \times W_1W_1ttepepMs_1Ms_1$
22	$w_1w_1ttepepms_1ms_1 \times W_1W_1TTEpEpMs_1Ms_1$
23	$w_1w_1Y_{11}Y_{11}TTms_1ms_1 \times W_1W_1Y_{11}y_{11}ttMs_1Ms_1$
24	$df_2df_2tt \times Df_2Df_2TT$

$$\left(\frac{1-p^2}{3}\right), \left(\frac{p^2}{3}\right), \left(\frac{2-2p+2p^2}{3}\right), \left(\frac{2p-2p^2}{3}\right).$$

The maximum likelihood equation for estimating recombination values from Weiss (1970) is

$$2a\left(\frac{1-p}{p(2-p)}\right) - b\left(\frac{2}{1-p}\right) - c\left(\frac{1-2p}{1-p+p^2}\right) + d\left(\frac{1-2p}{p(1-p)}\right) = 0.$$

The amount of information per individual, *i*, is obtained from the following equation:

$$\frac{4}{3}\left[\frac{1}{p(2-p)} + \frac{(1-2p)^2}{2p(1-p)(1-p+p^2)}\right].$$

The standard errors, S.E., for recombination values were calculated by the formula S.E. = $(1/I)^{1/2}$, where *I* is the total amount of information for all plants, *n*, classified.

For coupling data, the maximum likelihood equation used for estimating recombination values is

$$-a\left(\frac{2p}{1-p^2}\right) + b\left(\frac{2p}{p^2}\right) - c\left(\frac{1-2p}{1-p+p^2}\right) + d\left(\frac{1-2p}{p-p^2}\right) = 0.$$

The amount of information per individual, *i*, is obtained from the following equation:

$$\frac{4}{3}\left[\frac{1}{1-p^2} + \frac{1-4p+4p^2}{(2p-2p^2)(1-p+p^2)}\right].$$

The standard errors, S.E., for recombination values were calculated by the formula S.E. = $(1/I)^{1/2}$, where *I* is the total amount of information for all plants, *n*, classified.

For coupling phase linkage, the $F_{2:3}$ phenotypic classes involving Y_{11} and gene loci are $e = Y_{11}\ Y_{11}\ A\ A$, $f = Y_{11}\ Y_{11}\ A\ a$, $g = Y_{11}\ y_{11}\ A\ A$, and $h = Y_{11}\ y_{11}\ A\ a$ in a 1:2:2:4 ratio, where *A A*, is either W_1W_1, or Ms_1Ms_1, or Df_2Df_2, or *T T* and *A a* is either W_1w_1, or Ms_1ms_1, or Df_2df_2, or *T t*.

The expectations of these classes are, respectively,

$$\left(\frac{1-2p+p^2}{3-2p+p^2}\right), \left(\frac{2p-2p^2}{3-2p+p^2}\right), \left(\frac{2p-2p^2}{3-2p+p^2}\right), \left(\frac{2-4p-4p^2}{3-2p+p^2}\right).$$

The maximum likelihood equation for estimating recombination values is

$$-e\left(\frac{4-4p}{(1-2p-p^2)(3-2p+p^2)}\right) + f\left(\frac{3-6p+2p^2}{(p-p^2)(3-2p+p^2)}\right) + g\left(\frac{3-6p+2p^2}{(p-p^2)(3-2p+p^2)}\right) - h\left(\frac{4-10p+2p^2}{(1-2p+2p^2)(3-2p+p^2)}\right) = 0.$$

The amount of information per individual, *i*, is obtained from the following equation:

$$\left[\frac{(12-48p+60p^2-16p^3)}{(1-p^2)(9-12p-10p^2-4p^3+p^4)(1-2p+2p^2)}\right].$$

The standard errors, S.E., for recombination values were calculated by the formula S.E. = $(1/I)^{1/2}$, where *I* is the total amount of information for all plants, *n*, classified.

RESULTS AND DISCUSSION

Recombination values were calculated from classification of F_2 populations (Table 3) and $F_{2:3}$ families (Table 4), and from the combination of F_2 and $F_{2:3}$ data (Table 5). We did not obtain segregation data to enable estimation of linkage between St_5 - Adh_1, but Uncu (2001) reported a recombination value of 32%. From repulsion data, Weiss (1970) reported linkage between Y_{11} and Df_2, with a recombination frequency of 12%. Our F_2 and $F_{2:3}$ coupling data indicated linkage, although our values are greater (27 and 26%). Palmer and Chen (1998) also reported a recombination frequency of 25% from coupling data. The recombination frequencies among loci of CLG 8 are similar to those reported in the literature. Close linkages were observed for loci pair $Mm - W_1$, $W_1 - Ms_6$, and $Y_{23} - St_5$, with recombination frequencies of, 3, 4, and 3%, respectively. These values agreed with the values of 0 to 2.2% reported by others (Buzzell, 1975, 1976; Buzzell et al., 1977) for $Wm - W_1$, 2.5 to 5% reported by Ilarslan et al. (1999); Lewers and Palmer (1993); Palmer et al. (1998a); and Skorupska and Palmer (1989) for $W_1 - Ms_6$, and 1 to 7% reported by Lewers and Palmer (1993), and Palmer et al. (1990, 1998a) for $Y_{23} - St_5$. The recombination values in this study suggested moderately close linkages between $W_1 - Y_{23}$, St_5, Adh_1, and between $Ms_6 - Y_{23}$, St_5, and Adh_1, and confirm values reported by others (Bult et al., 1989; Kiang, 1990; Kiang and Chiang, 1987; Lewers and Palmer, 1993; Palmer and Kaul, 1983; Palmer et al., 1990).

Recombination values between $Df_2 - Ms_1$, $Df_2 - W_1$, and $Y_{11} - Ms_1$ suggested linkage, similar to linkages mentioned earlier among loci of CLG 8. Our F_2 data

Table 3. Percentage recombination values for loci pair calculated from F_2 soybean progeny data used for evaluating classical linkage groups 6 and 8.

Loci pair	Cross number†	A-B-	A-bb	aaB-	aabb	No. F_2 plants	χ^2 Deviation‡	% R ± S.E.§
W_1-Adh_1	3,4,5,19	1 785	255	263	479	2 782	823.9	20.2 ± 0.9
W_1-Ms_1	1,3,7,9,10,13	10 450	1 717	1 759	1 917	15 843	2 092.9	26.2 ± 0.4
W_1-Ms_1	6,8,16,20	3 421¶	1 482	1 530	154	6 587	301.7	30.3 ± 1.1
W_1-Ms_6	4,11,12,14	1 756	46	51	536	2 389	1 839.5	4.2 ± 0.4
W_1-St_5	10,11,18	872	108	112	227	1 319	428.4	18.2 ± 1.2
W_1-Y_{23}	5,13,14,18	4 886	555	522	1 089	7 052	2 067.8	17.3 ± 0.5
W_1-Df_2	1	835	194	254	139	1 422	43.0	38.6 ± 1.7
Adh_1-Ms_1	3	519	137	136	100	892	44.8	36.2 ± 2.1
Adh_1-Ms_6	4	589	65	74	155	883	305.9	17.3 ± 1.4
Y_{23}-Ms_1	13	1 073	243	300	140	1 756	31.4	40.2 ± 1.6
Y_{23}-Ms_6	14	1 690	147	142	409	2 388	943.9	13.4 ± 0.8
Y_{23}-St_5	18	836	15	16	280	1 147	1 031.4	2.7 ± 0.5
Ms_1-St_5	10	386	108	93	61	645	18.7	38.6 ± 2.6
Ms_6-St_5	11	238	14	22	67	341	175.1	11.2 ± 1.8
Ms_1-Df_2	1	944	145	195	138	1 422	106.2	30.2 ± 1.5
Y_{23}-Adh_1	5	368	19	18	120	525	378.3	7.2 ± 1.2
W_1-Wm	8	1 402	37	29	461	1 929	1 650.5	3.5 ± 0.4
Ms_1-Wm	8	589	235	234	20	1 078	42.0	29.4 ± 2.8
W_1-Y_{11}#	1,2,9,20	1 375	1 416	1 479	763	5 033	1 640.0	I
Ms_1-Y_{11}	1,9	869	131	1 236	214	2 450	92.5	31.7 ± 1.4
Ms_1-Y_{11}	20	419	168	936	303	1 826	4.0	46.4 ± 1.7
Df_2-Y_{11}	1	34	5	75	11	125	2.9	27.0 ± 5.9
W_1-T	2,3,5,9	4 479	1 512	1 474	489	7 954	0.1	I
Y_{23}-T	5	287	88	119	34	528	0.2	I
Ms_1-T	22	1 367	434	469	149	2 419	0.0	I
Adh_1-T	3,5	330	147	93	28	598	3.5	I
Df_2-T	24	432	147	144	50	773	0.01	I
T-Y_{11}	23	884	747	1 266	586	3 483	528.70	I
W_1-Y_9	7	693	220	244	80	1 237	0.04	I
W_1-Y_{10}	16	2 434	767	742	278	4 221	4.40	47.6 ± 1.1
Ms_1-Y_9	7	291	89	99	35	514	0.38	I
Ms_1-Y_{10}	16	577	139	155	45	916	1.21	I

† Cross number refers to crosses in Table 2.
‡ χ^2 tested deviation from 50% recombination; critical χ^2 (1 d.f., 0.05) = 3.84.
§ Percentage recombination ± standard error; I = independent assortment, i.e., R ≥ 50%.
¶ Repulsion data are underscored.
# Segregation ratio for Y_{11} − loci is $3Y_{11}Y_{11}A\text{-} : 1Y_{11}Y_{11}aa : 6Y_{11}y_{11}A\text{-} : 2Y_{11}y_{11}aa$.

Table 4. Percentage recombination values for loci pair calculated from $F_{2:3}$ soybean data used for evaluating classical linkage groups 6 and 8.

Loci pair	Cross number†	AABB	AABb	AaBB	AaBb	No. F_3 families	χ^2 Deviation‡	% R ± S.E.§
W_1-Ms_1	1,3,7,9,10,13	525	379	351	916	2171	375.57	28.3 ± 1.1
W_1-St_5	10,11	63	64	27	170	324	22.78	24.4 ± 2.6
W_1-Y_{23}	5,13	63	35	12	200	310	26.63	12.2 ± 1.7
W_1-Df_2	1,2,20	538	621	445	843	2447	293.01	36.4 ± 1.3
Y_{23}-St_5	18	405	16	16	238	675	1633.50	3.5 ± 0.6
Ms_1-Df_2	1,2	484	255	235	592	1566	621.34	24.8 ± 1.2
Ms_1-Df_2	15	34¶	87	97	200	418	3.75	41.6 ± 3.4
Ms_1-Ms_6	12	24	25	29	74	152	3.37	34.2 ± 5.0
W_1-Y_{11}	1,2,9	269	257	324	379	1229	144.51	38.4 ± 3.2
Ms_1-Y_{11}	1,2	235	155	204	319	913	197.81	32.0 ± 1.9
Df_2-Y_{11}	1,2,9,20	491	302	158	386	1337	888.06	25.9 ± 1.3
W_1-T	2,3,5,9	240	322	455	630	1647	19.97	I
Ms_1-T	22	34	65	62	106	267	0.71	I
Ms_1-T	3,9,21	141	328	236	623	1328	0.33	I
T-Y_{11}	23	76	80	142	176	474	11.63	I
Df_2-T	2	164	232	293	377	1066	19.71	I
Ms_1-Y_{18}	17	29	56	52	100	237	0.30	I

† Cross number refers to crosses in Table 2.
‡ χ^2 tested deviation from 50% recombination; critical χ^2 (1 d.f., 0.05) = 3.84.
§ Percentage recombination ± standard error; I = independent assortment, i.e., $R \geq 50\%$.
¶ Repulsion data are underscored.

suggested independent assortment between Y_{11} and W_1 while $F_{2:3}$ data indicated linkage ($R = 38$). These linkage relationships are similar to those reported by Mahama and Palmer (1998) and Palmer and Chen (1998).

The recombination values shown in this study suggested independent assortment between loci of both CLG 6 and 8 and *T* of CLG 1, Y_9 of CLG 14, and Y_{10}, and Y_{18} which are not assigned to any linkage group at this time (Tables 2, 3, and 4). This is in agreement with their placement on different linkage groups.

We observed variations in recombination values calculated from F_2 compared with $F_{2:3}$ data, between coupling and repulsion data, between values calculated by the same researcher in different experiments, and be-

Table 5. Percentage recombination values for loci pair calculated from combined F_2 and $F_{2:3}$ soybean data used for evaluating classical linkage groups 6 and 8.

Loci pair	No. F_2 plants	No. $F_{2:3}$ families	χ^2 Deviation†	% R ± S.E.‡
W_1-Ms_1	22 430	2 171	2 640.00	30.5 ± 0.4
W_1-St_5	1 319	324	431.45	22.3 ± 1.2
W_1-Y_{23}	7 052	310	2 080.34	17.6 ± 0.5
Y_{23}-St_5	1 147	675	2 470.92	3.7 ± 0.4
W_1-Df_2	1 422	2 447	303.97	36.4 ± 1.1
Ms_1-Df_2	1 422	1 984	577.21	28.6 ± 0.9
W_1-T	7 954	1 647	2.23	I
Ms_1-T	2 419	1 595	0.28	I
Df_2-T	773	1 066	11.34	I

† χ^2 tested deviation from 50% recombination; critical χ^2 (1 d.f., 0.05) = 3.84.
‡ Percentage recombination ± standard error; I = independent assortment, i.e., R ≥ 50%.

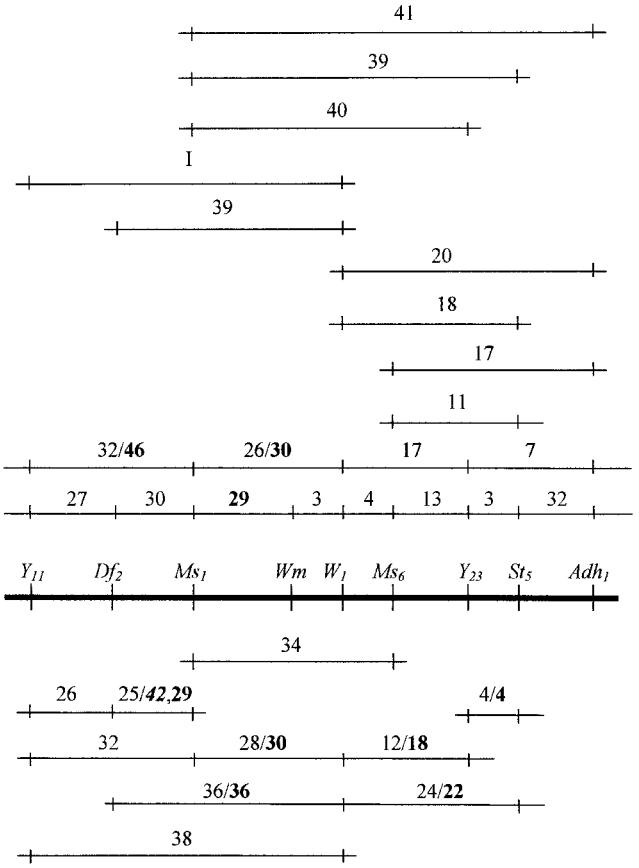

Fig. 1. Linkage map and orientation of loci of classical soybean genetic linkage groups 6 and 8. F_2 data (repulsion data in bold type) are above the chromosome segment (dark line) and $F_{2:3}$ (repulsion data in bold italic type) and combined F_2 and $F_{2:3}$ (bold type) data are below the chromosome segment. I means independent assortment.

tween values calculated by different researchers. Variation in recombination values has been documented in soybean (Griffin and Palmer, 1987; Palmer and Chen, 1998; Palmer et al., 1998b; Pfeiffer, 1993; Pfeiffer and Vogt, 1990). The recombination values, in general, are in close agreement with those from other reports, as they tend to place loci in the same order on the map. Such consistency confirms a consensus map proposed by Palmer and Hedges (1993).

Our recombination values suggest that, Df_2 and Y_{11} are linked to Ms_1 and W_1. This indicates that Df_2 and Y_{11} of CLG 6 are in the same linkage group as the Ms_1 and W_1 loci of CLG 8, and therefore on the same chromosome, with Y_{11} being distal to Ms_1 (Fig. 1). Comparing the recombination values between Y_{23} - St_5 and Y_{23} - Adh_1, the Adh_1 locus is distal to St_5. Recombination values between Ms_6 -Y_{23}, Ms_6 - St_5, Ms_1 –Y_{23}, and Ms_1 – St_5 suggest that, St_5 may be between Ms_6 and Y_{23}. Considering the standard errors of the recombination values of these loci pairs (Tables 3 and 4), and the recombination values between Ms_6 - St_5, and Ms_6 – Adh_1, however, the gene order Ms_6,Y_{23}, St_5, Adh_1 (Fig. 1) is favored.

CONCLUSIONS

On the basis of calculations of F_2, $F_{2:3}$, and combined F_2 and $F_{2:3}$ data, loci of classical linkage groups 6 and 8 assorted independently of loci of other linkage groups studied. The recombination values calculated in this study are in close agreement with reported values with the exception of the first reported (Weiss, 1970) recombination value calculated between Y_{11} and Df_2, of classical linkage group 6. Our data indicated linkage between Df_2 - Ms_1, Df_2 - W_1, Y_{11} - Ms_1, and Y_{11} - W_1, thus indicating that these loci belong to the same linkage group. Following integration of CLG 8 with MLG F and linkage between mutants of CLGs 6 and 8, and the satellite chromosome having been identified as chromosome 13, CLG 6, CLG 8, and MGL F are all on chromosome 13 of the soybean genome. The suggested orientation of loci is Y_{11} and Adh_1 at opposite ends of the chromosome segment studied, with Y_{23} between Ms_6 and St_5. The reassignment of CLGs 6 and 8 into one linkage group and the ordering of loci will facilitate their integration into and placement on the molecular map (Cregan et al., 1999; Shoemaker and Specht, 1995).

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